INVESTIGATION OF PORCUPINE BEZOAR EXTRACT (HYSTRIX BRACHYURAN) ON HeLa CELL COMBINED WITH ELECTROPORATION METHOD

AZRA HAZWANIE BINTI AZIZULKARIM

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ABSTRACT

Electroporation (EP) is a technique whereby the cell membrane is exposed to increase the intensity of electric field pulses that can lead to a variety of biophysical and biochemical responses. It is a molecular biology technique that stimulates pores through cell membrane, to increase the permeability of the cell, allowing chemicals, and drugs or Deoxyribonucleic acid (DNA) to be introduced into the cell. In this research, the HeLa cell behavior was investigated via EP. The purpose of this study is to investigate the best parameter of EP to be applied on HeLa cell treated with the best concentration of PBE which was 80.0ug/ml. Thus, the experiment is conducted for 48 hours and is initiated by identifying the best parameter for EP process. The range of electric field used is commenced between 100V/cm and 500V/cm with the increment of 100 V/cm interval sand for the pulse duration from 100µs, 500µs, 1ms, 2ms and 5ms. Based on the parameter used, the best result obtained is when the parameter was set to 500 V/cm for the electric field and 100µs for pulse duration, where it inhibits the lowest proliferation rate percentage of HeLa cell. For the natural resource compound or extract which is available in Malaysia Porcupine Bezoar (Hystrix Brachyuran) were used. This extract has the potential as an anti-proliferation agent, which is useful in anti-cancer application. The experiment suggests that the best concentration for Porcupine Bezoar Extract (PBE) was at 80.0ug/ml. This combined method of EP and PBE revealed the process inhibited the proliferation of HeLa cell to 8.48%. In comparison to the study without this combined technique of EP and PBE, the inhibition process reached 55.35%.The study has successfully demonstrated the contribution towards a new method for anti-cancer treatment due to its minimal side effects compared to other conservative methods such as Electrochemotherapy (ECT).

ABSTRAK

Elektroporasi adalah satu teknik di mana sel membran terdedah kepada peningkatan intensity medan elektrik yang boleh membawa kepada pelbagai tindak balas biofizik dan biokimia. Ini adalah teknik biologi molekular untuk menghasilkan liang melalui sel membran, untuk meningkatkan kebolehtelapan sel, membolehkan bahan kimia, dan dadah atau DNA diperkenalkan ke dalam sel. Dalamkajian ini, ciri-ciri sel HeLa dikaji menggunakan teknik elektroporasi. Objektif kajian ini adalah untuk mengkaji parameter elektroporasi yang terbaik untuk digunakan pada sel HeLa yang juga dirawat menggunakan kepekatan terbaik PBE iaitu pada kepekatan 80.0µg/ml. Oleh itu, eksperimen telah dilakukan selama 48 jam dan bermula dengan menentukan parameter terbaik bagi proses elektroporasi. Julat voltan yang digunakan bermula dari 100V/cm, 200V/cm, 300V/cm, 400V/cm, dan 500V/cm, manakala untuk tempoh denyut yang digunakan bermula dari 100µs, 500µs, 1ms, 2ms dan 5ms. Berdasarkan semua parameter yang digunakan, parameter terbaik yang diperoleh adalah bila disetkan pada 500V/cm untuk julat voltan, dan 100µs untuk tempoh denyut yang mana dapat menghasilkan peratusan kadar proliferasi terendah bagi sel HeLa. Selain itu, sebatian atau ekstrak sumber asli yang terdapat di Malaysia dikenali sebagai Hystrix Brachyuran yang telah digunakan. Ekstrak ini berpotensi sebagai ejen anti-proliferasi yang berguna dalam proses anti-proliferasi dalam kajian anti-kanser. Dari eksperimen ini, kepekatan terbaik PBEadalah pada 80.0µg/ml. Kaedah gabungan antara EP dan PBE ini membuktikan bahawa proses untuk menghalang percambahan sel HeLa kepada 8.48%. Berbanding dengan kajian tanpa teknik gabungan EP dan PBE ini, proses pencambahan mencapai 55.35%. Oleh itu kajian menunjukkan potensi yang sangat besar sebagai sumbangan terhadap kaedah baru untuk rawatan anti-kanser yang mana mempunyai kesan sampingan yang minimal berbanding dengan kaedah konservatif yang lain seperti elektrokimoterapi (ECT).

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LIST OF SYMBOL AND ABBREVIATIONS

DNA	-	Deoxyribonucleic acid
ECM 830	-	Square Wave Pulse Generator
ECT	-	Electrochemotherapy
EP	-	Electroporation
FBS	-	Fetal Bovine Serum
HeLa	-	Cervical Cancer Cell
IRE	-	Irreversible Electroporation
PBE	-	Porcupine Bezoar Extract
PBS	-	Phosphate Buffer Saline (PBS)
PEF	-	Pulse Electric Field
RE	-	Reversible Electroporation
RPMI 1640	-	Roswell Park Memorial Institute Medium
SEFPECT	-	Standard Electric Field Parameters for Electrochemotherapy
TPC	-	Total phenolic content
TFC	-	Total flavanoid content

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Cancer is one of the leading factors of human casualty which is approximate at 8.2 million globally per year and most likely to increase to 13 million worldwide per year until 2030 (Mehta *et al*., 2014). Moreover, oncology has become the biggest therapeutic area in the pharmaceutical sector in terms of the number of project clinical trials on research and development spending (Moffat *et al.,* 2014).

The past decade has been an escalation in cancer biology research with many new promising disease targets. However, the capability to translate these advances into therapies are poor, with a failure rate of about 90% (Adams *et al.,* 2012). Budget reduction on studies and development by pharmaceutical companies and expenditure increment of bringing a new molecular entity to market, the anti-drug advancement had becomes a highly complex problem (Adams *et al.,* 2012). Moreover, advances in cancer cure have proved in high number of long – term cancer survivors whom most of them were left to experience the side effect from their treatments such as hair loss, joint pain and nausea. One of the contributing factor includes the drugs intake during treatment. For over 50 years, the investigation of anti-cancer drugs has been directed by the fact that tumour cell replicates more expeditiously than normal cell and that DNA is the most essential element in cell division. DNA is a common therapeutic

target of anti-cancer drugs, and most of the currently available drugs may cause DNA damages, interrupts cell division and finally causes cell death (Bailón-Moscoso *et al.*, 2014).

Studies of anti-cancer, by using animal extract as a chemotherapeutic agent are investigated rapidly. The use of Porcupine Bezoar Extract (PBE) in this research will be explored of its capability to inhibit the proliferation rate percentage of HeLa cell through Electroporation (EP). This is accomplished by using EP with animal extract. Bioactive extract from porcupine bezoar which is also known as the "prince of antidotes" (Barroso MD and Duffin CJ, 2013), that has the capabilities of anti-proliferation and also anti-angiogenesis properties for cancer growth is employed. Porcupine bezoar or porcupine stone is extracted from porcupine rodents with a coat or sharp quills, which normally will defend and camouflage themselves from predators like snakes or tigers. Since conventional treatment often cause certain side effect to cancer survivors, PBE will be explored to whether it reduces or inhibit the proliferation rate percentage of HeLa cell through EP technique. This could serve as an alternative method for cancer treatment as it originates naturally and, with minimal or no side effects on its application.

1.2 Problem statement

Presently, cancer is known to be the leading cause and factor of death throughout the world. It is one of the major threats to public health in both developed and developing nations. In developed countries, cancer is the second most common cause of death (Moffat *et al.*, 2014). Studies show that cancer is caused by both external and internal factors. The external factors are tobacco, consumption, radiations, chemicals, and infectious organism whilst internal factors include inherited mutations, hormones and also immune condition related to sedentary lifestyles likes smoking, poor diet and inactive physical activities (Mehta *et al*., 2014).

Various treatments are provided in order to help cancer patients, for instance chemotherapy, immune treatment, radiation therapy, hyperthermia, hormone therapy and many more. Consequently, advances in cancer therapy will lead and result in increasing numbers of long-term survivors who are left to themselves to handle the

consequences of their treatments. Therefore, this research is organised as an effort to explore alternative treatment by using EP technique and by focusing on alternative agent to suppress, delay, or reverse carcinogenesis using pharmacologic intervention with naturally occurring or synthetic agents.

1.3 Aim and Objectives

The aim of this research is to investigate the technique and bioactive extract that are able capable to inhibit cancer cell growth (anti-proliferation) by using EP technique and animal extract of PBE test on cultured HeLa cell properties.This study embarks on the following objectives:

1) To investigate the suitable parameters of EP technique for Reversible Electroporation (RE) process on Hela cell.

2) To analyze the effect of PBE on HeLa cell lines for anti-proliferation process.

3) To evaluate the anti-proliferation mechanism by combining both PBE and the optimized EP method for maximum HeLa cell anti-proliferation activity.

1.4 Scopes of study

In order to achieve the objectives of this research, the following scope of work have been identified such as the process of growing and plating HeLa cell on the surface, expose to pulse electric field (PEF), and to test the PBE efficiency:

1. HeLa cell was cultured in an environment similar to that of the host tissue. The cell were sterile, controlled and maintained at temperature of 37ºC and humidified environment at 5% carbon dioxide (Co_2) in order to maintain

the pH, the scale used to specify how acidic or basic a water-based solution of the medium similar to pH of the human blood plasma.

2. Investigation of the best EP parameter for the inhibition growth of HeLa cell in-vitro. EP parameters used:
   i. Electric field : 100 V/cm – 500 V/cm followed by Standard Electric Field Parameters for Electrochemotherapy (SEFPECT)
   ii. Pulse Duration : 100 µs – 5 ms.
   iii. No of pulses: Single pulse

3. Observation and examination of cellular behavior of HeLa cell in the presence of PBE with exploration of lower extract concentration ranging from 10.0 µg/ml – 160.0 µg/ml. Moreover, half maximal inhibitory concentration, IC_{50} was measured to indicate how much concentration of PBE extract (inhibitor) is needed to inhibit the proliferation rate percentage of HeLa cell by half. Therefore, IC_{50} represents the concentration of PBE that is required for 50% inhibition in-vitro of HeLa cell's proliferation rate percentage.

4. The experiments were performed thrice for consistency, and the mean or average proliferation factor was then measured.

1.5 Thesis structure:

The rest of the thesis is structured into eight chapters as follows:

Chapter 2: This chapter covers a background and literature review on EP method. In addition, a brief description of HeLa cell and its natural behaviour/ characteristic are explained in this chapter. Moreover, general information regarding PBE and its benefits use in anti-proliferation application are also discussed in this chapter.

Chapter 3: This chapter provides a list of general materials and equipment used in carrying out the research. Detailed description of the general methods used in achieving the research aim is discussed in this chapter.

Chapter 4: In this chapter, the influence of EP method induced on HeLa cell line and the morphological changes of the cell is investigated, observed and reported.

Chapter 5: In this chapter, the most suitable electric field parameter for the growth of HeLa cell is investigated. The suitability of EP parameters is crucial and is applied in the subsequent chapters to study the influence of EP method on cellular behaviours of the cell and PBE.

Chapter 6: This chapter investigates the effect of PBE on the cellular behaviour of HeLa cell such as the anti-proliferation of the HeLa cell.

Chapter 7: In this chapter, the combined influence of EP and PBE on HeLa cell proliferation rate percentage towards anti-proliferation application are investigated and described.

Chapter 8: This final concludes the thesis and includes recommendations for future work.

CHAPTER 2

LITERATURE REVIEW

2.1 The Cell

Cells are the building blocks of life and are the smallest structural unit and functional organism of all living things (Rodamporn, 2011). The cells are derived from two types of pre-existing cell and managed to accomplish all the activities of cell. In addition, the cells are categorized into two groups, prokaryotic and eukaryotic cell (Rhoads, 2007). An example of a prokaryotic cell is a bacteria cell as shown in Figure 2.1. Eukaryotic cells include plant, animal, fungi and protist (Maskarinec *et al.*, 2006). Contrarily, the size of eukaryotic cells in Figure 2.2 is bigger compared with prokaryotic cells which are smaller in size and it have no nucleus. The cell wall membrane is very flexible with a thin layer surrounding the cell. The cell wall has a thick layer outside the cell membrane and that makes it very tough. The function of this cell wall is to give a physical rigidity and also will allow cellular material and chemical substance to pass through it.

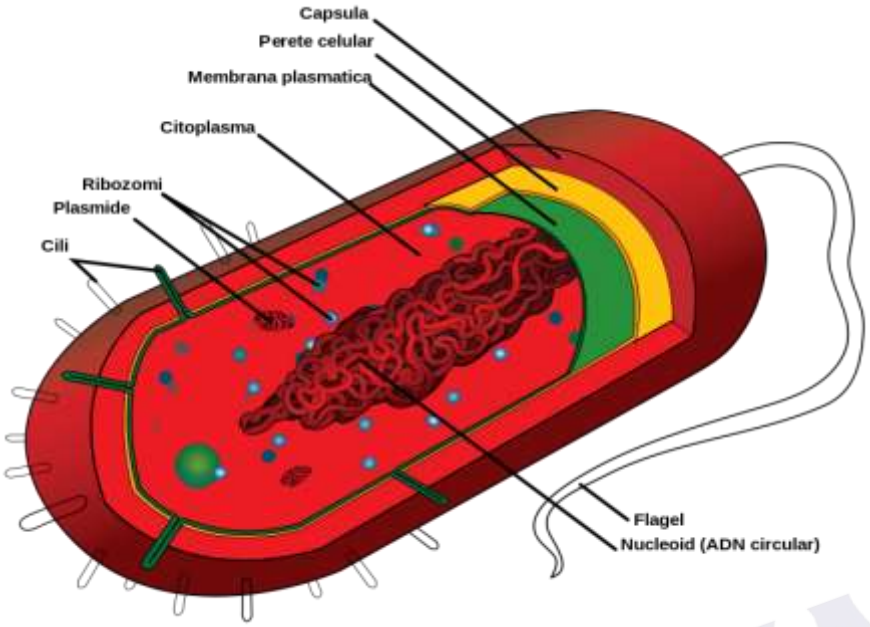

Figure 2.1: A diagram of a Prokaryotic cell
(https://commons.wikimedia.org)

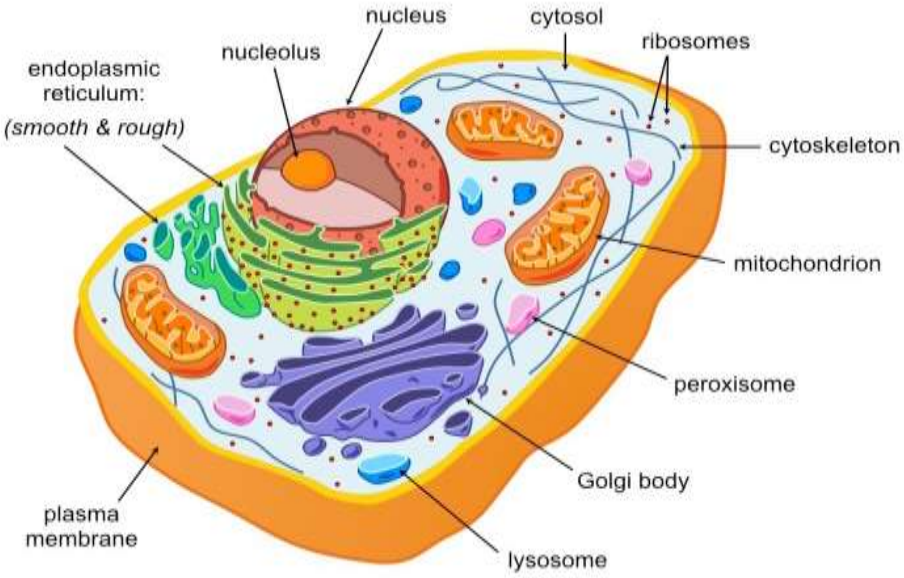

Figure 2.2: Eukaryotic cell. Adapted from
(http://ib.bioninja.com)

2.2 Composition of cell

2.2.1 Nucleus

One of the most important organelles in a cell is nucleus. Nucleus found in eukaryotic only cells and has two main functions: it retains and stores the cell's hereditary information or genetic information through (DNA); and coordinates and controls cell's activities, which include intermediary metabolism, cell division, protein synthesis, and also cell growth (Will W. Minuth and Raimund Strehl, 2005). The nucleus is composed of a double membrane nuclear envelope that carries the entire organelle sheathed, and the nucleoskeleton that helps in supporting the cell as a whole. The nucleus also controls the safety of the genes and command the activity of the entire cell by managing the gene expression. Thus, the nucleus is referred as the control centre or also known as the brain of the cell (Will W. Minuth and Raimund Strehl, 2005). Figure 2.3 shows the image of cell nucleus.

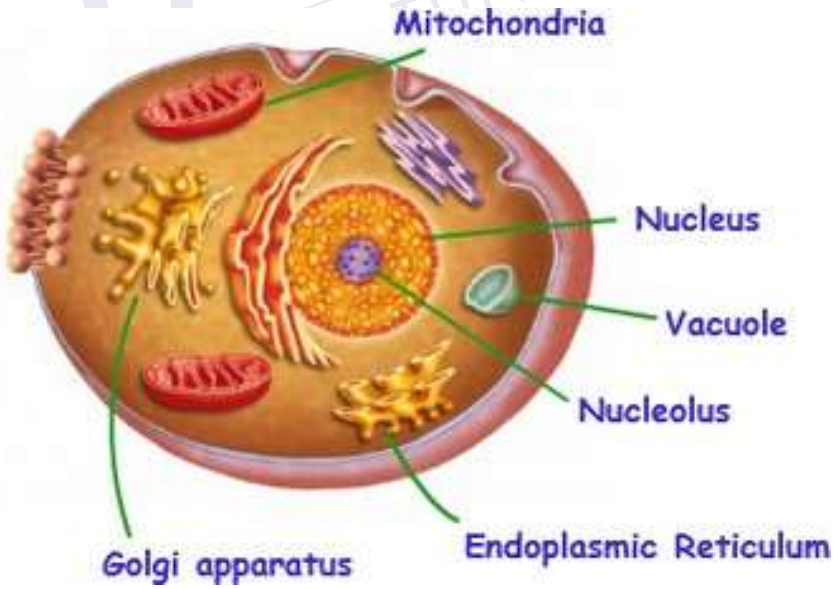

Figure 2.3: Illustration of Nucleus. Adapted from (https://biology.tutorvista.com)

2.2.2 Cell membrane

Cell membrane or also known as plasma membrane (Figure 2.4) acts as a semi-porous barrier to the external environment. The cell membrane is not only the boundary of the cell unit, but it also has a specific compartment that accommodates many essential cell functions, carrying molecules in and out of cell and helps in metabolic functions (Lombard, J., 2014). In addition, cell membrane allows the cell to communicate with its external surroundings. Hydrophobic tails and hydrophilic heads are the composition of the cell membrane known as phospholipid bilayer. Other than phospholipids, cholesterols are also present in the cell membrane. Cell membrane only allows certain substances to diffuse into it like oxygen (O_2), carbon dioxide (Co_2) and water (H_2O) while ions and some polar molecules can't diffuse through the bilayer because the membrane are made up from lipids due to existence of stability and flexibility in lipids bilayers.

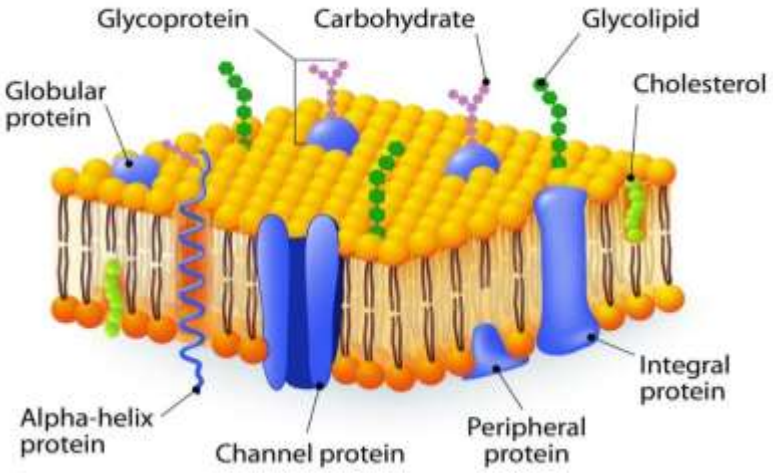

Figure 2.4: Illustration of Cell Membrane. Adapted from (http://anatomysciences.com)

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